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A comparative SEM study of morphological characters in *Cribraria*

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Abstract—*Cribraria* spp. were examined by SEM to determine detailed morphological features of the spore, nodes, and calyculus and to identify and describe the characters and character states for each microstructure examined. The character states defined include six for spore ornamentation (warted, crested, spiny, subreticulate, banded-reticulate, grooved-reticulate), three for node types (rounded, enlarged, irregular), and four for the upper calyculus margin (ribbed, irregular, toothed, entire). Our SEM studies show that interpretations of spore ornamentation character states based solely on LM may be incorrect and that SEM observations are crucial for discovering new characters and interpreting taxonomic morphological features.

Key words—*Cribrariaceae*, morphology, myxomycetes, plasmodial granules, slime molds

Introduction

Myxomycetes (now formally classified by www.indexfungorum.org in *Myxogastrea*) are a small, relatively homogeneous group of eukaryotic organisms with small fructifications (sporangia) and a spore size range of 5–15 µm (Stephenson & Stempen 1994). The ornamented spore surface is very difficult to assess using only light microscopy (LM) (Singer et al. 2006), and the magnification and resolution limitations of LM prevent close examination of the fine structure of sporangia (Hatano 1985). Lane (1985) proposed that scanning electron microscope (SEM) techniques be used to describe new characters and to assist in the proper interpretation and taxonomic use of previously unknown features.

Spore ornamentation, size, and (in some species) the way in which the dehydrated spore wall collapses are among the most useful diagnostic characters for identifying myxomycete species. The ability of SEM to produce taxonomically significant information and to resolve minute details has proved superior to other forms of microscopy in visualizing important diagnostic characters (Schoknecht & Small 1972, Lyon & Kramer 1977). Although Gaither & Collins (1984) credited Schoknecht & Small as the first to emphasize SEM for resolving taxonomical questions in myxomycete systematics, Keller & Reynolds (1971) had previously described a new species of *Perichaena* with the aid of the SEM images. Subsequently several other authors have used SEM techniques to identify new taxonomic characters (Ellis et al. 1973, Rammeloo 1974, Eliasson & Sunhede 1980, Nelson et al. 1982) and to provide more inclusive descriptions of new species (Keller & Reynolds 1971, Keller et al. 1975, Mulleavy 1977, Raub et al. 1979, Whitney & Olive 1983).

Cribraria species produce some of the smaller sporangia (up to 1 mm in *C. zonatispora*), which in some instances are detectable only when the substratum is observed microscopically at 50–100× or when the specimens are produced in moist chamber cultures. Lado (2017) currently recognizes 46 species of *Cribraria*. Morphological characters that vary among species—type of nodes, surface spore ornamentation, calyculus margin, and nature of the peridial net—are among those most frequently used in taxonomic descriptions. In the literature, most species descriptions are based on LM observations; to date only 18 *Cribraria* species have been described with SEM details (Hatano 1985, Keller et al. 1988, Lado et al. 1999, Estrada-Torres et al. 2001, Moreno et al. 2001, Hatano 2002, Liu & Chang 2007, Hooff 2009). Hatano (1985), in a study of myxomycetes from Japan, provided SEM details for ten species in the *Cribrariaceae* (*Cribraria argillacea, C. aurantiaca, C. cancellata, C. cribrarioides, C. intricata, C. languescens, C. microcarpa, C. minutissima, C. rufa,* and *C. tenella*), although the spores were collapsed in *C. minutissima* and *C. rufa;* Keller et al. (1988), who discussed the systematics of *C. minutissima* and *C. confusa,* included SEM photographs with detailed spore morphology and noted that the spores tended to collapse along preformed lines over the surface. Lado et al. (1999) described *C. zonatispora* as having 'unique' spores, while Estrada-Torres et al. (2001) described unique spore ornamentation for their new species, *C. fragilis,* from México. In their contribution to the myxomycetes of Baja California (México), Moreno et al. (2001) described the spore ornamentation as observed under SEM for *C. intricata, C. purpurea,* and *C. violacea*. Hatano (2002) studied and described the surface ornamentation of the sporangium, calyculus, peridial surface net and spore ornamentation for five species from the Korean Peninsula (*C. intricata, C. microcarpa, C. rufa, C. splendens* and *C. tenella*). More recently, Liu & Chang (2007) described *C. angulospora* and Hooff (2009) described *C. tecta* using characters observed under SEM.

In a general spore classification scheme for all myxomycetes, Hatano (1985) proposed seven types of spore ornamentation, including *Cribraria* in type I (warts) with four subtypes (a, b, c, d). Each subtype was defined based on the frequency or distribution of warts on the spore surface. Although he considered warts the predominant type of ornamentation in *Cribraria* spores, a completely different surface ornamentation has been observed under SEM in some *Cribraria* species, e.g. *C. zonatispora*, *C. fragilis*, and, *C. tecta* (Lado et al. 1999, Estrada-Torres et al. 2001, Hooff 2009).

In view of the morphological variability of the spore surface and other taxonomically important structures found in *Cribraria*, the objective of our study was to contribute to knowledge of the genus through SEM examination and to identify potentially informative morphological characters and character states of the microstructures that will eventually help reveal phylogenetic relationships within the genus.

Materials & methods

Taxon sampling

We obtained specimens representing 35 (of 46 known) *Cribraria* species from the U.S. National Fungus Collection, Beltsville MD, U.S.A. (BPI), the herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS, now donated to herb TLXM), and the myxomycete collection at the herbarium of the Universidad Autónoma de Tlaxcala, México (TLXM). Whenever more than two specimens per species were obtained, we processed the most abundant and typical (based on our comparison with the descriptions) collection for SEM observation. Here we describe only SEM observations for 21 species, as we were unsuccessful in processing the specimens representing the other 14 species.

Processing of the sporangium for SEM

Because material collected in the field is generally dehydrated when preserved in a herbarium, preparation for SEM observation required rehydration of the samples for eight hours in a 1:1 solution of concentrated ammonium hydroxide and distilled water. After rehydration, samples were dehydrated in a graded

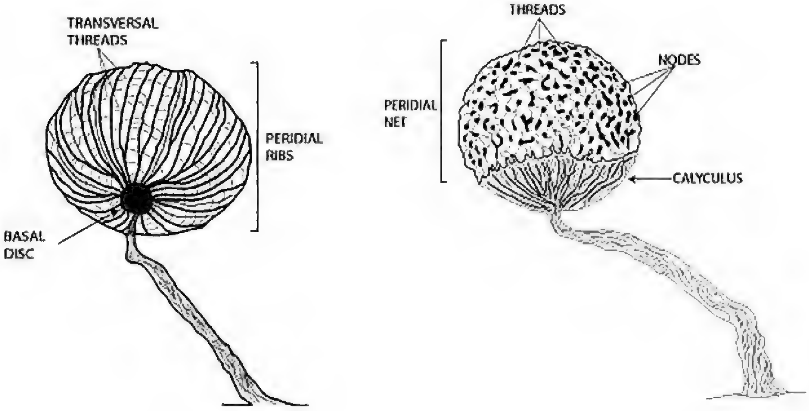

FIGURE 1. Morphological sporocarp structures described for *Cribraria* species.

ethyl alcohol series of 10, 20, 30, 50, 90 and 100% concentrations and then acetone solution for ten minutes at each step. The samples were then dried using a critical point technique, mounted on specimen stubs with double face adhesive tape, and coated with gold/ palladium in a high-vacuum chamber with a JFC-1100 Jeol Fine Coat Ion Sputter. The samples were examined with a JEOL JSM-5600 scanning electron microscope.

Results

The characters targeted under the SEM include (1) the peridium, (2) the type of node, (3) the margin and outer surface of the calyculus, and (4) the spore surface. Below, we detail the taxonomic characters for *Cribraria* as observed under SEM and briefly compare them with the characters described by previous authors (based on SEM and LM). For a better understanding of the morphological terms used in this paper, we also provide a drawing depicting important generic features in *Cribraria* (FIG. 1) and a brief glossary (p. 419).

Species & SEM descriptions

Cribraria atrofusca G.W. Martin & Lovejoy,
J. Wash. Acad. Sci. 22: 92, 1932. PLATE 1a–d

SPECIMEN BPI 828412—PERIDIUM: consisting of an upper net and a shallow cup at the base of the sporotheca. NODES: scarcely thickened, mostly elongated, connected by broad threads, sometimes with 2–3 free ends and forming angular isodiametric meshes. PLASMODIAL GRANULES: concentrated principally in the nodes but also in the inner side of the threads. Calyculus: membranous, shallow, usually well developed, about one third the total height of the entire sporotheca, the margin bearing fine long tooth-like projections, the outer calyculus surface characterized by pronounced concentric folds. Spores: (SEM images slightly collapsed) surface ornamentation apparently composed of a reticulum formed by thick ribs, with 4–5 meshes on one hemisphere and with warts, sometimes fused and forming ridges, in the inner areas formed by the meshes of the reticulum.

Comments: The microstructure under SEM has not been previously reported for *C. atrofusca*. Martin & Alexopoulos (1969) described the spore ornamentation as "finely verrucose with faint, broken reticulate pattern" as seen by LM. Although most spores were collapsed in our study, we did observe that a ribbed-reticulate pattern and warted spore surface occur in this species. The concentric folds in the calyculus and the peridial net are the most distinctive features of this species.

Cribraria aurantiaca Schrad. Nov. Gen. Pl.: 5. 1797. Plate 1e–h

Specimen TNS 2612—Peridium: partially fugacious, consisting of a net occupying most of the sporotheca and leaving a calyculus in the base. Nodes: isodiametric, most rounded, thickened, connected by threads (c. 2 µm diam.) forming a regular mesh. Plasmodial granules: (c. 2 µm diam.) concentrated in the nodes. Calyculus: membranous, shallow, usually well developed, about one-third the total height of sporotheca, margin characterized by ribs connecting with peridial net, upper surface with delicate concentric folds, radial folds at the base of the calyculus. Spores: globose, 5–6 µm diam. with a surface ornamentation formed by warts; frequently several fuse to form an irregular ridge.

Comments: A SEM study by Hatano (1985) reported *C. aurantiaca* with a spore ornamentation composed of many warts, sometimes adhering to each other and forming irregular ridges. Our observations of the spore ornamentation, node, and calyculus characters agree with Hatano.

Cribraria cancellata (Batsch) Nann.-Bremek.,
Nederlandse Myxomyceten 92. 1975. Plate 2a–c

Specimen TLXM Rodríguez-Palma 2191—Peridium: partly evanescent, remaining as 50–60 ribs connected by fine transverse threads. Plasmodial granules: distributed along the rib surfaces, 1–2 µm diam. Spores: globose, c. 4–5 µm diam.; surface ornamentation composed of winding crests, sometimes branched and forming a subreticulate pattern. STALK: grooved and attenuate at the apex. SPOROTHECA: oblate, sometimes globose, usually umbilicate at the apex, generally nodding, rarely erect.

COMMENTS: Martin & Alexopoulos (1969) and Lado & Pando (1997) described the spore surface as smooth or minutely punctate, but irregular and winding crests are easily seen with SEM. Hatano (1985), who described the spore ornamentation in the same way, referred to *C. cancellata* as having vein-like warts. We did not see a basal disc or calyculus in our specimen but did observe that the ribs extend from the stalk apex.

Cribraria costata Dhillon & Nann.-Bremek.,
Proc. Kon. Ned. Akad. Wetensch., C 81: 141. 1978. PLATE 2d–h

SPECIMEN TNS 16898—PERIDIUM: partially evanescent, remaining as ribs that arise from the base to almost the top of the sporotheca, connected by perpendicular threads, forming a net at the upper part of the sporangium. NODES: pulvinate, isodiametric, connected by strip-like threads that form an open mesh and with few free ends. PLASMODIAL GRANULES: concentrated in the nodes. CALYCULUS: (if present) a basal disc. SPORES: globose, 6–7 µm diam.; surface ornamentation composed of winding crestae, sometimes branched and forming a subreticulate pattern.

COMMENTS: The specimen we studied fits well the original description given by Dhillon & Nannenga-Bremekamp (1978). The microstructure as seen by SEM has never been reported before for *C. costata*. The ribs radiating from the apex of the stalk and the pyriform shape of the sporotheca are the distinctive characters for this species.

Cribraria dictyospora G.W. Martin & Lovejoy,
J. Wash. Acad. Sci. 22: 91. 1932. PLATE 3a–d

SPECIMEN TNS 2936—PERIDIUM: with a shallow basal cup and a fine meshed net in the upper portion of the sporotheca. NODES: flattened and elongated, with narrow connecting threads with few free ends. CALYCULUS: shallow, occupying one-third the total height of the sporotheca, marked with radial folds on the outer surface, margin toothed. SPORES: polyhedric, 5–6 µm diam., surface ornamentation composed of a wide meshed ribbed reticulum, giving a polyhedric appearance of the spores, with 4–5 meshes per spore side and warts in the inner surface of the meshes. STALK: erect and striate. SPOROTHECA: globose or subglobose, erect to slightly nodding.

COMMENTS: Although the spores seem to be slightly collapsed, the surface spore ornamentation is clearly formed by a reticulate pattern with fine warts inside the meshed areas. These are the first SEM observations of *C. dictyospora.*

Cribraria intricata Schrad. Nov. Gen. Pl. 7. 1797. PLATE 3e–h

SPECIMEN BPI 828726)—PERIDIUM: partially evanescent, in most of the sporotheca remaining as a net but sometimes also as a basal disc or cup. NODES: thickened, elongated, roughened, connected by ≤8 slender threads with two or three free ends. PLASMODIAL GRANULES: concentrated on the outer side of the nodes. CALYCULUS: shallow when present, with the upper margin extending into ribs, which are connected with the peridial net; surface with prominent radial folds marked by the concentration of the plasmodial granules and fine concentric folds. When the calyculus is absent, subparallel ribs emerge from the stalk apex. SPORES: globose, 4–5 µm diam., with the surface ornamentation formed by warts; frequently several fused to form an irregular ridge.

COMMENTS: Hatano (1985, 2002) mentions that under SEM the spore surface of *C. intricata* has many warts with corrugate swellings. Although in our specimen most spores were collapsed, our SEM observations appear closer to those described by Hatano in 1985; our specimen showed a perfectly developed calyculus, contrasting with the observation by Hatano in 2002 in which the calyculus was reduced to a basal disc and the ribs emerged from the base of the sporotheca. As Martin & Alexopoulos (1969) stated, *C. intricata* is very variable in these features.

Cribraria languescens Rex,
Proc. Acad. Nat. Sci. Philadelphia 43: 394. 1891 PLATE 4a–c

SPECIMEN TLXM ET44090—PERIDIUM: partially fugacious, remaining as a calyculus at the base and as a net in the upper portion. NODES: pulvinate, isodiametric, connected by 5–6 threads that form an irregular open mesh with few free ends. PLASMODIAL GRANULES: concentrated on the nodes. CALYCULUS: shallow, up to half of the sporotheca, with nearly even or somewhat toothed margins, with faint radial folds. SPORES: globose, 6–7 µm diam., with the surface ornamentation formed by remarkably dense, 0.1–0.2 µm diam. warts, several frequently fused to form irregular and sometimes branched ridges. STALK: long, slender, slightly grooved.

COMMENTS: The surface ornamentation of the spore was described as densely warted, with the warts forming irregular ridges (Hatano 1985). Our study corroborates the observations of *C. languescens* by this author.

Cribraria laxa Hagelst. Mycologia 21: 298. 1929. PLATE 4d–h

SPECIMEN BPI 828894—PERIDIUM: partially evanescent, consisting of ribs that radiate from the stalk apex, a membranous calyculus at the sporothecal base, and a very lax net in the upper portion. NODES: isodiametric, sometimes elongated, pulvinate, connected by 4–5 slender threads, with 1–2 free ends, basal ribs with not connecting threads between them. CALYCULUS: irregular, shallow, formed by thin membrane between the basal ribs, with an irregular margin. SPORES: globose, 5–6 µm diam., with the surface ornamentation formed by 0.3–0.5 µm long spines, frequently fused to form irregular ridges in a sub-reticulate pattern.

COMMENTS: The spiny ornamentation of the spore in *C. laxa* forms a subreticulate pattern, seemingly unique in *Cribraria.* Although Martin & Alexopoulos (1969) described a net with elongated and large nodes, we also frequently observed rounded nodes.

Cribraria lepida Meyl, Bull. Soc. Vaud. Sci. Nat. 56:326. 1927. PLATE 5a–c

SPECIMEN BPI 828797—PERIDIUM: partially evanescent, remaining as a prominent and deep basal calyculus and an open net at the upper portion of the sporotheca. NODES: irregular, pulvinate, densely covered with plasmodial granules, connected by 4–5 thin threads, without free ends. CALYCULUS: deep, up to half of the sporotheca, margin even, outer surface granulose due to the presence of the plasmodial granules. SPORES: globose, 6–7 µm diam., with the surface ornamentation formed by warts that frequently fuse to form irregular short ridges. SPOROTHECA: globose or cylindrical, nodding.

COMMENTS: The spores of *C. lepida* have been described as smooth using LM (Martin & Alexopoulos 1969), but they are clearly warted with SEM. Although this species is similar to *C. violacea* in color, size, habit, and even the warted spore ornamentation, the nodes differ considerably: flattened in *C. violacea* and pulvinate in *C. lepida*. As seen with SEM, in *C. lepida* the nodes resemble a cluster of grapes due to the aggregation of plasmodial granules.

Cribraria macrocarpa Schrad. Nov. Gen. Pl.: 8. 1797. PLATE 5d–h

SPECIMEN BPI 828967)—PERIDIUM: partially fugacious, remaining as a shallow calyculus at the base and a dense net with very close meshes in the upper part. NODES: irregular, large and elongated, flattened, connected by 4–5 wide threads with membranous margins forming a close-meshed net, with 2–3 free ends. PLASMODIAL GRANULES: 2 µm diam., smooth, distributed on the nodes and on the whole calyculus. Calyculus: occupying one-third the sporotheca, well developed, margin irregular with many perforations in the upper portion, with radial folds on outer surface. Spores: globose, 6–7 µm diam., with the surface ornamentation formed by warts, frequently fused to form irregular short ridges. Stalk: wide and slightly grooved. Sporotheca: globose or ovate, nodding or erect.

Comments: The calyculus with the perforated margin and the flattened and irregular nodes confirm the identity of *C. macrocarpa,* based on the LM description by Martin & Alexopoulos (1969). Adamonyte & Eliasson (2010) compared it within a species complex comprising *C. martinii, C. argillacea,* and *C. ferruginea*, which all share a perforated peridial calyculus. Nonetheless, we have no SEM evidence confirming this similarity of *C. macrocarpa* with the other species.

Cribraria microcarpa (Schrad.) Pers., Syn. Meth. Fun.:190. 1801. Plate 6a–d

Specimen BPI 828978—Peridium: partially fugacious, remaining as ribs at the base of the sporotheca and a net above; basal ribs not interconnected with fine threads, emerging from the apex of the stalk. Nodes: small, pulvinate, hemispherical or sometimes elongated, studded with plasmodial granules, connected by faint threads that form a close net with polygonal meshes and without free ends. Calyculus: absent. Spores: globose, 6–7 µm diam., with the surface ornamentation formed by 0.1 µm diam. spines. Sporotheca: globose, erect or nodding. Stalk: slender, the apex thinner than the rest of the stalk, plicate.

Comments: Hatano (1985) cited two types of spore ornamentation in *C. microcarpa*—one with warts and the other with spines. Our observations agree with Hatano's second choice, even though our spores were not completely round. Martin & Alexopoulos (1969) and Hatano (1985) observed that when present, the calyculus is reduced to a minute basal disc. Our SEM images clearly show that the basal disc is lacking but that ribs, which radiate from the apex of the stalk and connect with the peridial net, are present. Nannenga-Bremekamp (1993) described this feature as "threads springing from the stalk."

Cribraria mirabilis (Rostaf.) Massee, Monogr. Myxogastr.: 60. 1892. Plate 6e–h

Specimen TLXM GF44—Peridium: partially evanescent, remaining as sub-parallel ribs from the base to the middle of the sporotheca, connected by delicate threads and an irregular net in the upper portion. Nodes: flattened, irregular, elongated, connected by 4–5 threads (similar in width to the basal ribs) with no free ends. Plasmodial granules: distributed along the ribs. Calyculus: not observed. Spores: globose, 5–6 µm diam., surface ornamentation composed of winding crests, sometimes branched to form a subreticulate pattern. Sporotheca: globose, erect or nodding.

Comments: *Cribraria mirabilis* has never previously been published based on SEM observation. SEM observation revealed that the spore ornamentation is composed of minute crests that sometimes fuse to give the appearance of ridges. Our SEM images also show that the ribs extend from the base of the sporotheca to form a well-developed net in the upper part.

Cribraria oregana H.C. Gilbert, Amer. J. Bot. 19:142. 1932. Plate 7a–c

Specimen TLXM HC183—Peridium: partially evanescent, remaining as a basal shallow calyculus and an irregular net in the upper portion. Nodes: flattened, irregular, connected by 4–5 threads, which form irregular meshes and open net with few free ends. Calyculus: shallow, with irregular margins and smooth surface. Spores: globose, 7–8 µm diam., with the surface ornamentation formed by minute and dense warts, frequently fused to form irregular short ridges. Sporotheca: globose, usually erect. Stalk: slender, slightly attenuated at the apex, longitudinally striate.

Comments: This is the first SEM description for *C. oregana*. Relying on LM, Martin & Alexopoulos (1969) described the spore ornamentation as "minutely warted, with a delicate reticulum" while Lado & Pando (1997) defined it as "spinulose." Our SEM examination revealed the absence of both reticulum and spines and the presence of abundant minute warts that fuse to form irregular short ridges.

Cribraria piriformis Schrad. Nov. Gen. Pl.: 4. 1797. Plate 7d–h

Specimen BPI 829249—Peridium: partially fugacious, remaining as a calyculus at the base of the sporotheca and dense net in the upper portion. Nodes: isodiametric to elongated, pulvinate, depressed on the inner side, covered with plasmodial granules, connected by 3–7 diam. threads that form a closed net and with 3–6 rounded free ends and wider tips. Calyculus: shallow, with irregular margins and with some rib-like projections connecting the peridial net and with pronounced concentric folds on the outer surface. Spores: globose, 6–7 µm diam., with the surface ornamented by abundant minute warts in aggregates. Sporotheca: globose to pyriform, erect. Stalk: furrowed, slightly attenuated at the apex.

Comments: Ours is the first SEM description for *C. piriformis*. It has thickened nodes as well as wide connecting threads in the peridial net. Nodes on the inner side are markedly hollow (Pl. 7f). Under LM some authors (Martin & Alexopoulos 1969, Lado & Pando 1997, Adamonyte & Eliasson 2010) have described the spore ornamentation as densely warted, and our SEM images confirm this character.

Cribraria purpurea Schrad. Nov. Gen. Pl.: 8. 1797. Plate 8a–d

Specimen TLXM HC1869—Peridium: partially evanescent, remaining as a shallow basal calyculus and a net in the upper portion. Nodes: flattened, irregular in size and form, sometimes undifferentiated, connected by 3–6 very fine threads, with few free ends. Plasmodial granules: hollow, on the inner side of nodes, 2–3 µm diam., smooth. Calyculus: shallow, with irregular or toothed margin, with concentric folds. Spores: globose, 5–6 µm diam., with surface ornamentation composed of winding crests, sometimes branched and forming a subreticulate pattern. Sporotheca: globose, nodding.

Comments: *Cribraria purpurea* was described using SEM by Moreno et al. (2001). However, our examination revealed a different spore ornamentation composed of crests fused to form a sub-reticulate pattern, not observed in the image provided by Moreno et al. (2001, Fig. 21), who described the ornamentation as "baculate" (a completely different pattern). It would be interesting to confirm that both examined specimens represent *C. purpurea*.

Cribraria rubiginosa Fr. Syst. Mycol. 3:172. 1829. Plate 8e–h

Specimen TLXM ET2720—Peridium: partially fugacious, remaining as a deep basal cup and a loose net occupying the upper half of the sporotheca. Nodes: irregular, flattened, somewhat angular, filled with plasmodial granules and connected with strip-like threads, without free ends. Plasmodial granules c. 1–2 µm diam. Calyculus: deep, with irregular margins, radial folds covered with plasmodial granules at least at the base of the sporotheca. Spores: globose, 6–7 µm diam., ornamented by a prominent reticulum formed by grooves, with four meshes per side, and pronounced warts in the meshes, sometimes fused. Sporotheca: cylindrical, erect. Stalk: grooved.

Comments: Martin & Alexopoulos (1969) describe the spores as angular and with a warted ornamentation by LM. Our SEM image shows a very well defined reticulum formed by grooves that cross the whole spore surface and warts between the meshes of the reticulum, in a similar way found on the spores of *C. angulospora*, a species recently described from Taiwan (Liu & Chang 2007). Nevertheless we suspect that *C. angulospora* may represent a synonym of *C. rubiginosa*. Martin & Alexopoulos (1969) mentioned that the angular shape of the spore depends on the angle in which the spore is observed in the microscope, but it is clear that this angularity is due to the presence of the reticulum.

Cribraria rufa (Roth) Rostaf. Śluzowce monogr.: 232. 1875. PLATE 9a–d

SPECIMEN BPI 829339—PERIDIUM: partially evanescent, remaining as a shallow basal calyculus and an open-meshed net in the upper portion of the sporotheca. NODES: flattened, irregular to elongate connected by 3–5 wide threads, without free ends. CALYCULUS: short, irregular margin, radially plicate ribs, extending from the base of the sporotheca and connecting with the peridial net, outer surface with very marked radial folds. SPORES: globose, somewhat angular, 7–8 µm diam., surface ornamentation composed of an open reticulum formed by thick ribs with warts in the inner areas formed by the meshes of the reticulum, the warts sometimes fused and forming short ridges. SPOROTHECA: globose or ovate, erect. STALK: erect, longitudinally striate.

COMMENTS: In a SEM study of *Cribrariaceae* from Korea, Hatano (2002) reported the spore ornamentation in *C. rufa* as a "corrugate swelling [that] connects each other and makes a vein-like protuberance." Our SEM observation of the spore surface shows a lax reticulum divided by spaces with warts included. Under LM Nannenga-Bremekamp (1991) described the spore ornamentation as having warts often connected by rows of smaller warts into a lax reticulum. Our observations agree with the description of spore ornamentation given by Nannenga-Bremekamp (1991). The angular appearance of the spore, as mentioned by Martin & Alexopoulos (1969), may be due to the presence of a lax reticulum on the spore surface.

Cribraria splendens (Schrad.) Pers., Syn. Meth. Fung.:191. 1801. PLATE 9e–h

SPECIMEN TLXM GF1769—PERIDIUM: partially fugacious, remaining as 4–5 thick ribs at the base and an open net in upper portion. NODES: flattened, not well differentiated, occasionally elongated, without free ends and connected by 3–4 fine threads, which have membranous margins and form an irregular and open meshed net; ribs radiating from the stalk and connecting with the peridial net. CALYCULUS: absent. SPORES: globose, 5–6 µm diam., with the surface ornamentation formed by warts, sometimes fused to form irregular short ridges. SPOROTHECA: globose, nodding. STALK: slender, curved at the apex, longitudinally striate.

COMMENTS: Hatano (2002) described the spore ornamentation for *C. splendens* under SEM as "corrugate swellings" that sometimes are fused and then a vein-like protuberance are observed. Our SEM and his photographs agree, but we prefer to use the more appropriate term "warts" for this type of ornamentation, noting that sometimes the warts fuse to form irregular short ridges.

Cribraria tenella Schrad. Nov. Gen. Pl.: 6. 1797. PLATE 10a–d

SPECIMEN TLXM ET4832—PERIDIUM: partially fugacious, remaining in the basal part of the sporotheca as a calyculus, and as a net in the rest. NODES: pulvinate, rounded, few free ends, connected usually by four slender threads. Net irregular, meshes open, with wide connecting threads. CALYCULUS: shallow, the margin irregular, surface smooth, sometimes radial folds emerging from the base, marked by the presence of plasmodial granules. SPORES: globose, minutely warted, the rest of the surface smooth, 5–6 µm diam. SPOROTHECA: globose, nodding.

COMMENTS: Hatano (2002) reported the spore surface "with protuberances" for this *C. tenella*. In general the peridial net and spore descriptions by Hatano (1985; 2002) correspond our results, except for the calyculus, which seem more regular and shallow in Hatano's description and more irregular and deeper in our SEM plate.

Cribraria violacea Rex, Proc. Acad. Nat. Sci. Philadelphia 43:393. 1891. PLATE 10e–h

SPECIMEN BPI 829575—PERIDIUM: partially evanescent, remaining as a deep calyculus at the base and as a net in the upper portion. NODES: flattened, irregular in size and form, with hollow protuberances due to the plasmodial granules, connected by delicate threads, which form an open net, with few and irregular meshes, without free ends. CALYCULUS: deep, occupying half or two thirds of the sporotheca, margin toothed, with the surface studded with plasmodial granules. SPORES: globose, 6–7 µm diam., with the surface ornamentation formed by warts, sometimes fused forming irregular short crests. SPOROTHECA: globose or cylindrical, erect. STALK: slender, grooved.

COMMENTS: Moreno et al. (2001) are the only authors to have described this species as "baculate with small crests" using SEM. The two SEM images are similar, but our SEM plate shows warts that are much closer and form irregular short crests. Under LM most authors have described *C. violacea* as minutely warted (Martin & Alexopoulos 1969, Nannenga-Bremekamp 1991, Lado & Pando 1997).

Cribraria vulgaris Schrad. Nov. Gen. Pl.: 6. 1797. PLATE 11

SPECIMEN BPI 829689—PERIDIUM: partially fugacious, remaining as a calyculus at the base and as a net in the upper portion of the sporotheca. NODES: irregular or isodiametric, flattened, connected by 4–5 threads that form an irregular net without free ends. CALYCULUS: occupying about one third of the lower portion of the sporotheca, with irregular margins, forming spiny teeth that give rise to the net, the surface marked by delicate radial folds. SPORES: 5–6 µm diam., the surface ornamented by warts that sometimes fuse to form irregular short ridges (not possible to confirm in collapsed spores). SPOROTHECA: globose, nodding. STALK: erect, slightly attenuate at the apex, longitudinally striate.

COMMENTS: Martin & Alexopoulos (1969) described the spore ornamentation of *C. vulgaris* based on LM as "minutely punctate;" nevertheless Nannenga-Bremekamp (1991), Lado & Pando (1997), and Adamonyte & Eliasson (2010) described it as "slightly angular, warted with faint ridges or with wartlets forming a lax reticulum." Warts are present and distributed on the spore surface in our SEM photographs, but the presumed reticulum was not observed due to the collapsed spore.

Character coding under phylogenetic perspective

Character description plays such an essential role in the phylogenetic inference process, determination of characters and character states is one of the most fundamental steps in any taxonomic work (Kirchoff 2001). Newton & De Luna (1999) noted, "we need not ascertain a priori if a character or its states have a common origin, or if character states are homologous" because these result from the cladistics analysis. The final result from the inference cladistics process must serve as the foundation for any discussion about evolutionary character change that will allow us to corroborate or change the homology hypothesis based on strict cladistic principles (Wirkner & Richter 2010).

In a phylogenetic context, Sereno (2007) defined the logical basis for morphological characters, where he concluded that "characters are simply features expressed as independent variables and character states

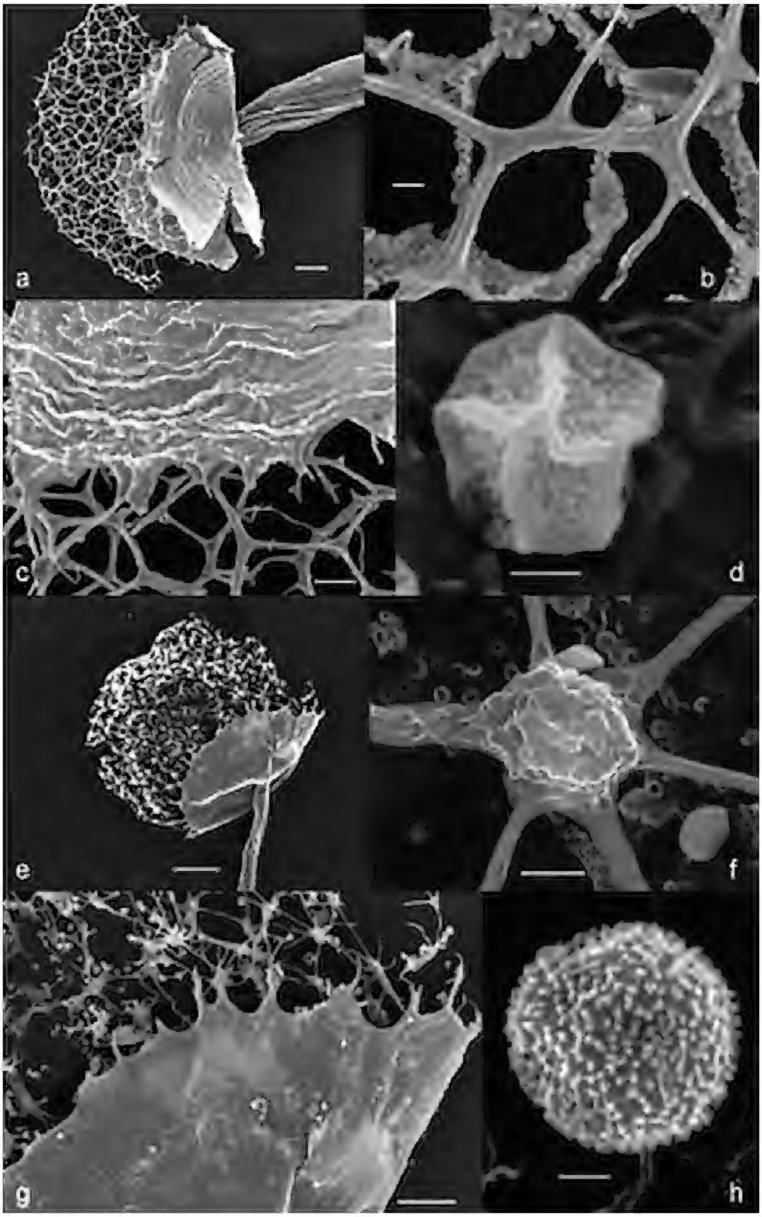

PLATE 1. ***Cribraria atrofusca***. a. Sporocarp; b. Enlarged node with plasmodic granules; c. Margin and outer surface of calyculus; d. Collapsed spore. ***Cribraria aurantiaca***. e. Sporocarp; f. Node; g. Margin and outer surface of calyculus; h. Surface spore ornamentation. Scale bars: a, e = 100 µm; b = 10 µm; c = 20 µm; d = 2 µm; f = 5 µm; g = 50 µm; h = 1 µm.

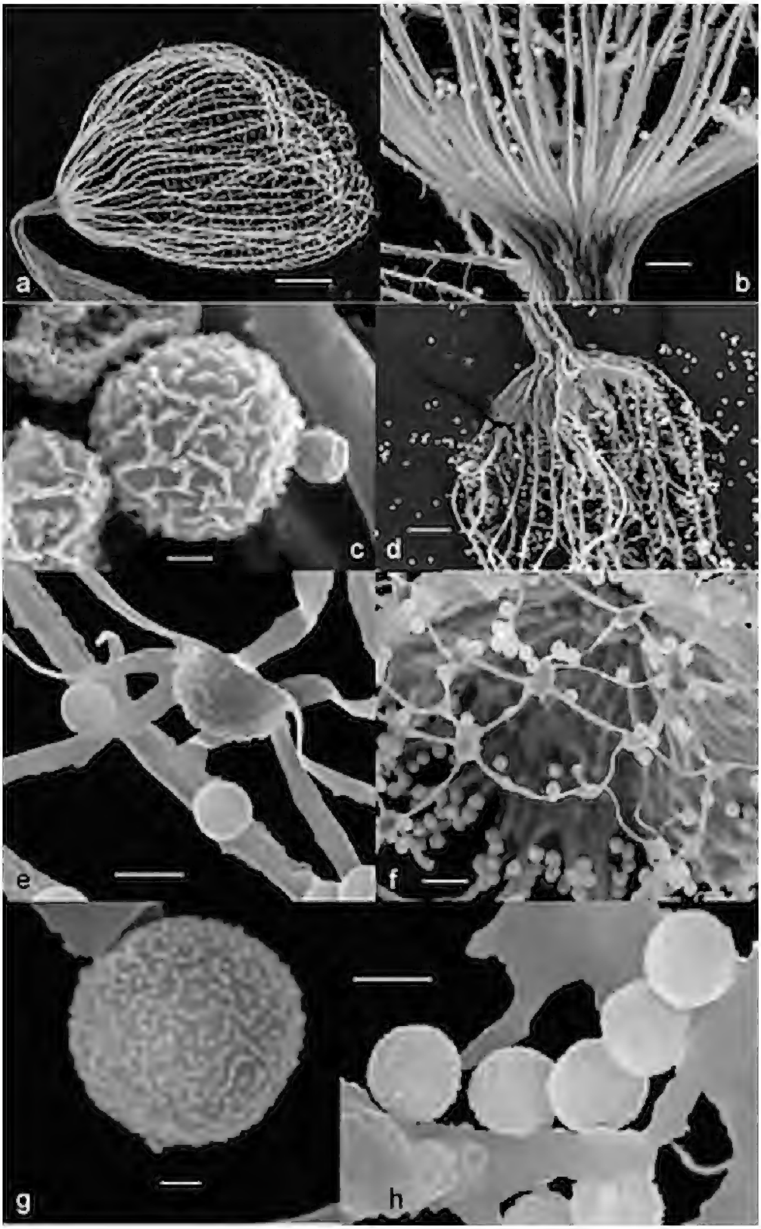

Plate 2. ***Cribraria cancellata***. a. Sporocarp; b. Peridial ribs emerging from the stalk apex; c. Spore and plasmodic granule. ***Cribraria costata***. d. Sporocarp; e. Node of peridial net; f. Peridial net without free ends; g. Spore surface ornamentation with crestae; h. Spores. Scale bars: a = 100 µm; b, f = 20 µm; c, g = 1 µm; d = 50 µm; e =10 µm; h = 5 µm.

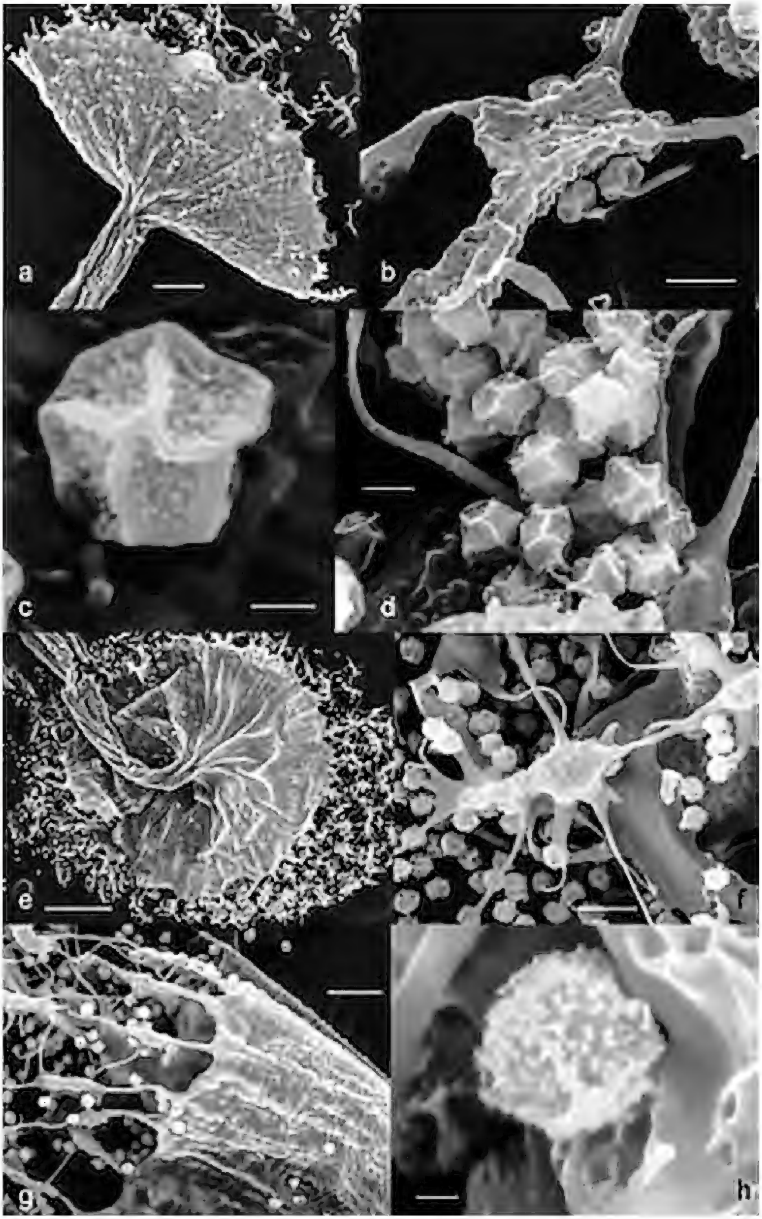

PLATE 3. ***Cribraria dictyospora***. a. Margin details and outer surface of calyculus; b. Node; c. Collapsed spore with reticulum; d. Collapsed spores showing surface reticula. ***Cribraria intricata***. e. Sporocarp; f. Peridial node with 2–3 free ends; g. Calyculus margin connecting with the peridial net and details of the outer surface; h. Spore surface ornamentation with warts. Scale bars: a = 50 µm; b, f = 10 µm; c= 2 µm; d = 5 µm; e = 100 µm; g = 20 µm; h = 1 µm.

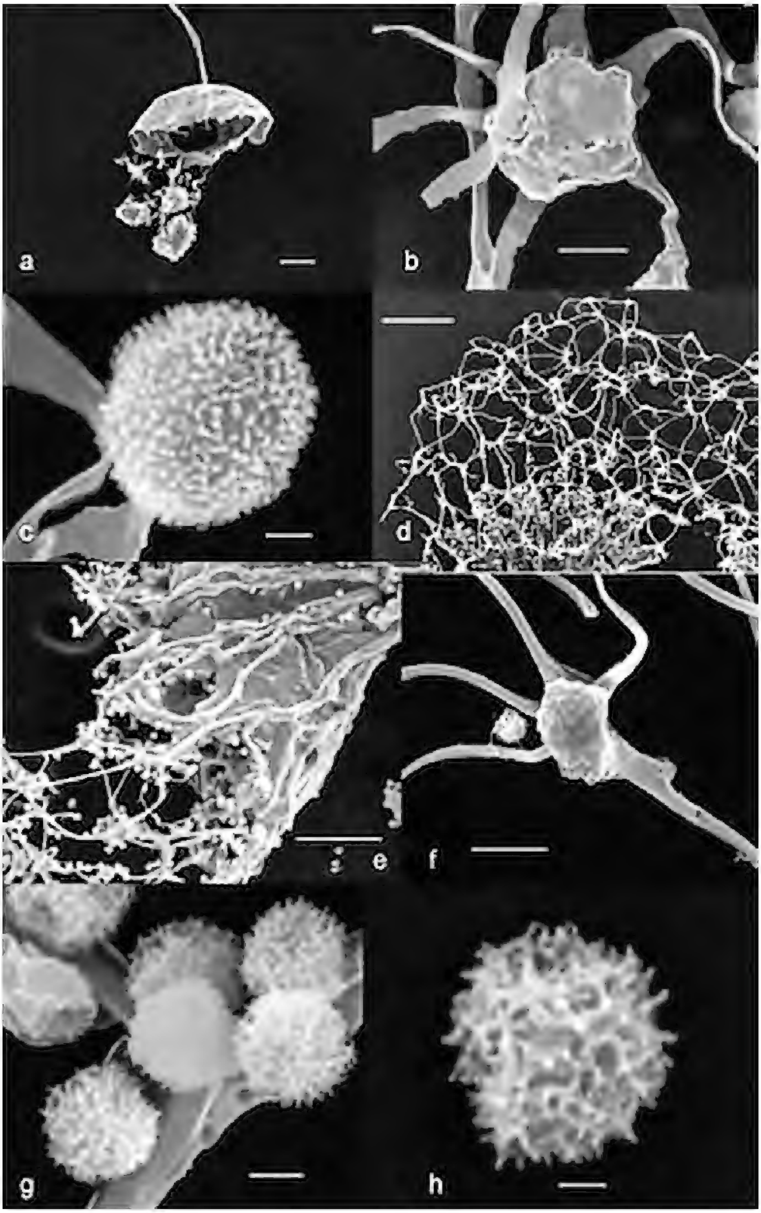

Plate 4. ***Cribraria languescens***. a. Sporocarp; b. Peridial node apparently without free ends; c. Spore surface ornamentation with warts. ***Cribraria laxa***. d. Peridial net; e. Peridial ribs emerging from the base of the sporocarp and details of the calyculus surface; 50 µm. f. Thickened node; g. Spores; h. Spore ornamentation. Scale bars: a, e = 50 µm; b = 5 µm; c, h = 1 µm; d = 100 µm; f = 10 µm; g = 2 µm.

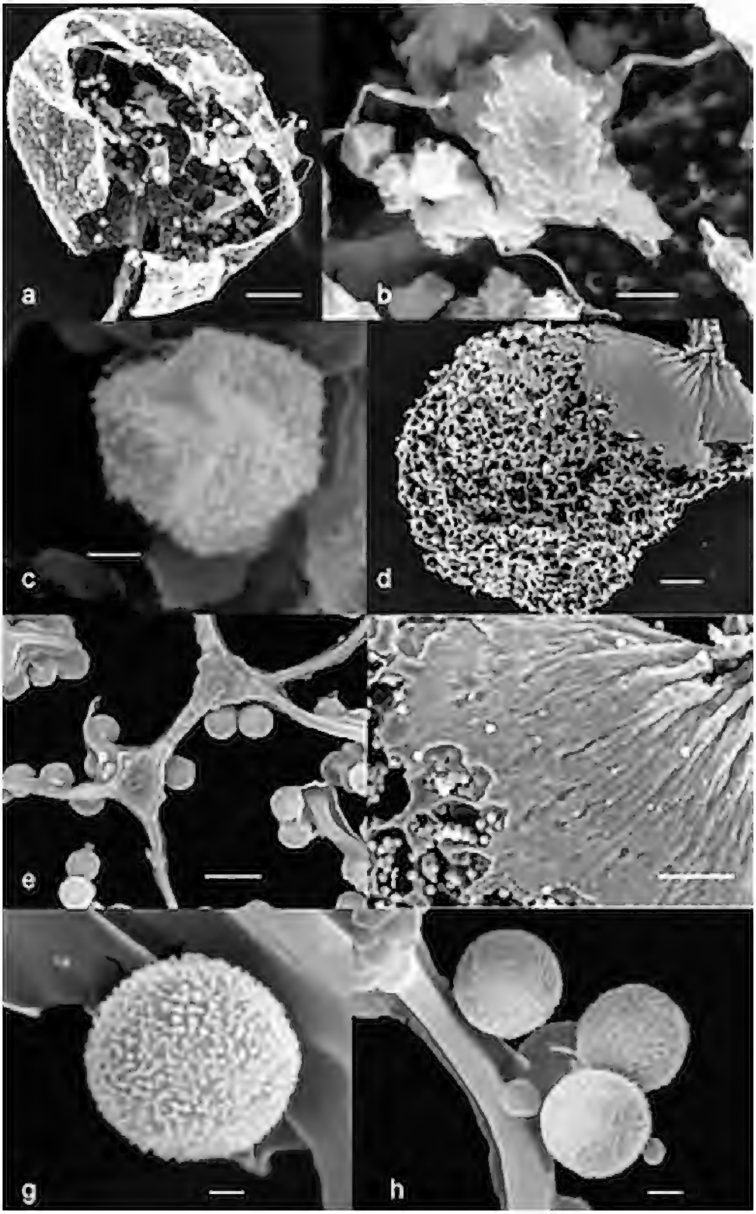

Plate 5. ***Cribraria lepida***. a. Sporocarp; b. Peridial node; c. Spore. ***Cribraria macrocarpa***. d. Sporocarp showing the peridial net and calyculus; e. Peridial node with plasmodic granules and spores; f. Details of calyculus outer surface; g. Spore surface ornamentation; h. Spores and plasmodic granules. Scale bar: a = 20 µm; b = 5 µm; c, g = 1 µm; d = 100 µm; e = 10 µm; f = 50 µm; h = 2 µm.

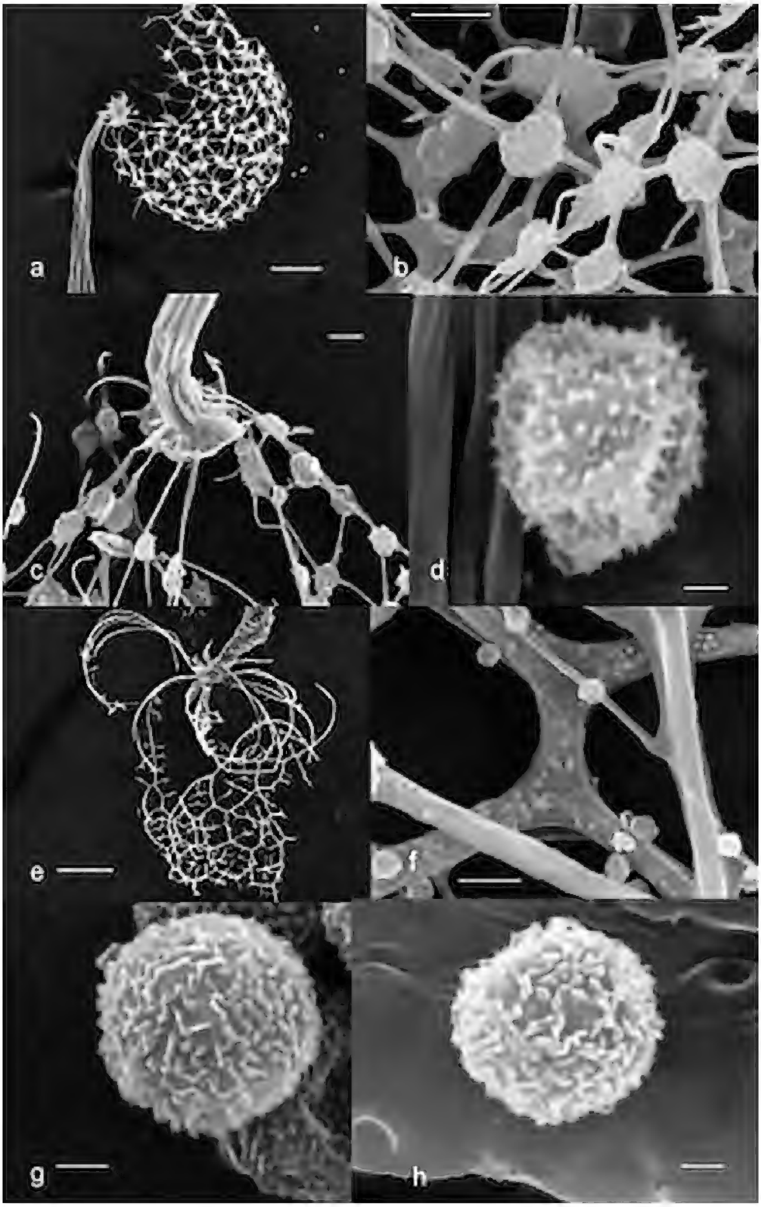

Plate 6. ***Cribraria microcarpa***. a. Peridial net of the sporocarp; b. Peridial nodes with plasmodic granules; c. Peridial ribs emerging from the apex of the stalk; d. Spore with spines as surface ornamentation. ***Cribraria mirabilis***. e. Sporocarp showing the peridial ribs and net; Peridial net without well-defined nodes; g. Spore surface ornamentation; h. Spore. Scale bars: a = 50 µm; b, c, f = 10 µm; d, g, h = 1 µm; e = 200 µm.

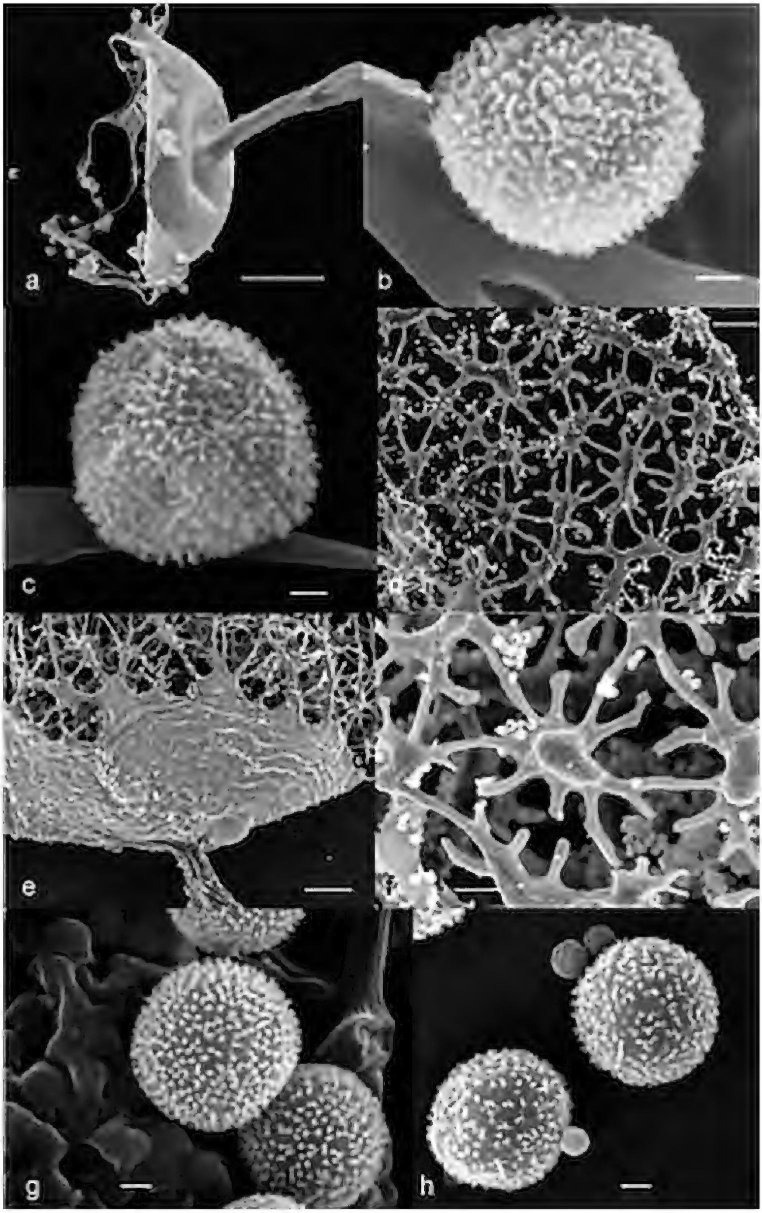

Plate 7. ***Cribraria oregana***. a. Sporocarp; b. Spore surface ornamentation; c. Spore with warts. ***Cribraria piriformis***. d. Peridial net and spores; e. Outer surface of calyculus and margin connecting with the peridial net; f. Peridial node with 2–3 free ends; g. Spores; h. Spores and plasmodic granules. Scale bars: a, d = 50 µm; b, c, g, h = 1 µm; e = 100 µm; f = 20 µm.

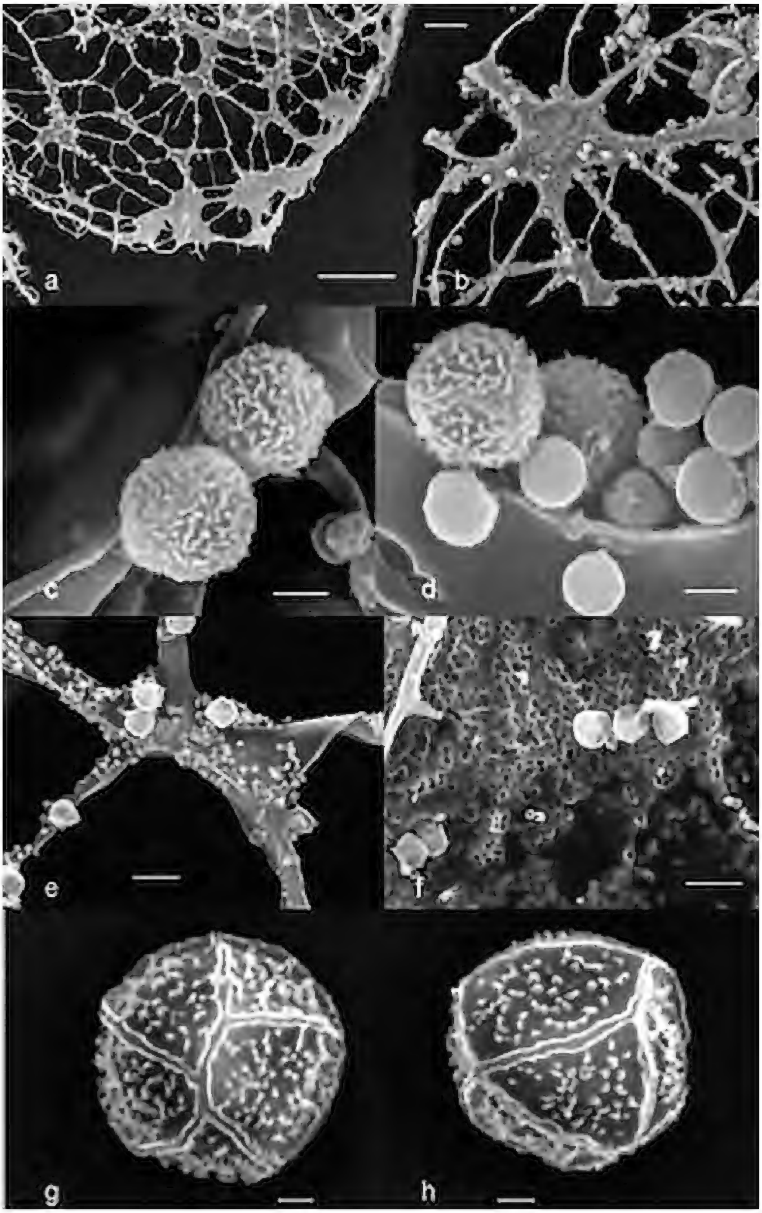

PLATE 8. ***Cribraria purpurea***. a. Peridial net; b. Nodes flattened without free ends; c. Spores with surface ornamentation composed of crests; d. Spores and plasmodic granules. ***Cribraria rubiginosa***. e. Flattened node with spores and plasmodic granules; f. Inner surface details of calyculus; g. Spore details of surface ornamentation showing warts and a reticulum groove crossing the spore; h. Spore seen in angular view. Scale bars: a =100 µm; b = 20 µm; c, d = 2 µm; e, f = 10 µm; g, h = 1 µm.

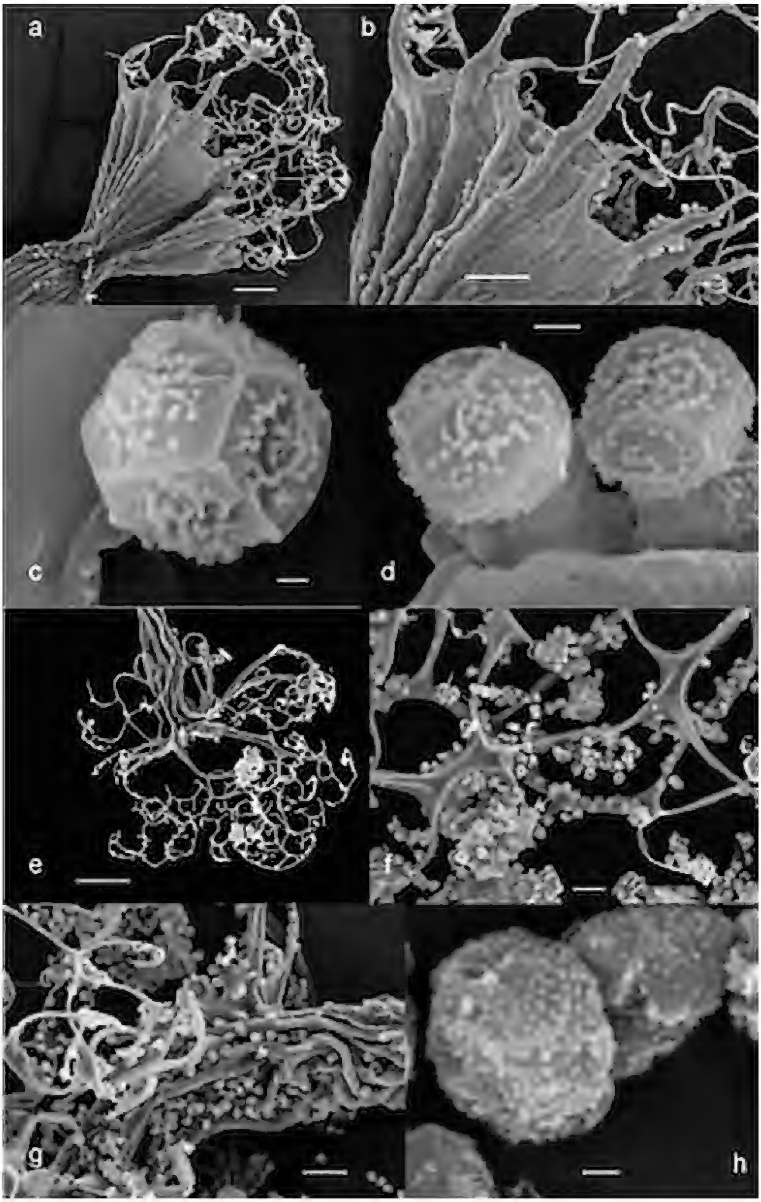

Plate 9. ***Cribraria rufa***. a. Sporocarp; b. Peridial ribs extending from the base of the sporotheca; c. Spore showing a reticulum and warts in the inner area; d. Spores. ***Cribraria splendens***. e. Sporocarp; f. Node flattened and elongated; g. Peridial ribs extending from the apex of the stalk; h. Spore surface ornamentation with warts. Scale bars: a, e =100 µm; b = 50 µm; c, h = 1 µm; d = 2 µm; f, g = 20 µm.

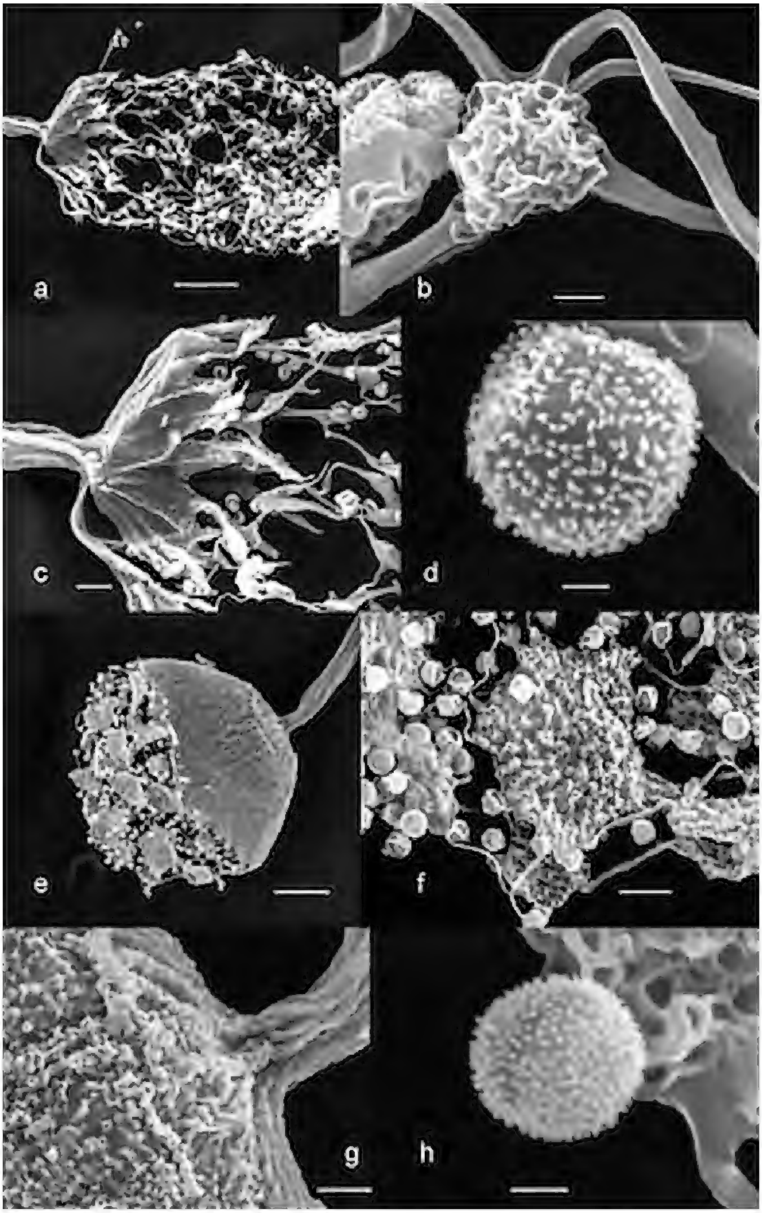

Plate 10. ***Cribraria tenella***. a. Sporocarp; b. Pulvinate and rounded node; c. Calyculus with radial folds emerging from the base; d. Spore surface ornamentation with warts. ***Cribraria violacea***. e. Sporocarp; f. Peridial node with hollow protuberances caused by plasmodic granules; g. Calyculus surface (detail); h. Spore with surface ornamentation formed by warts. Scale bars: a, e =50 µm; b, h = 2 µm; c, f, g = 10 µm; d = 1 µm.

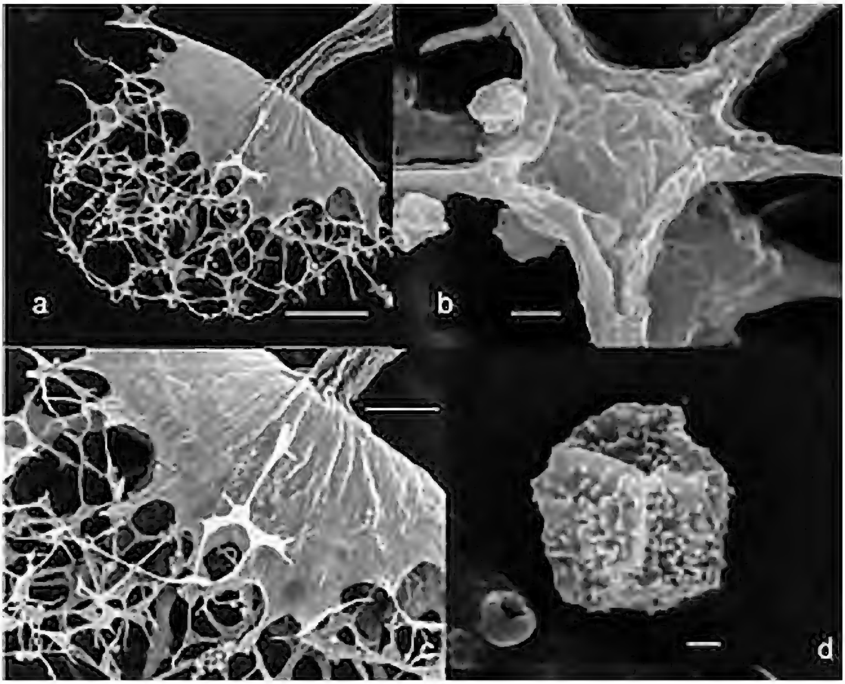

Plate 11. ***Cribraria vulgaris***. a. Sporocarp; b. Peridial node flattened and without free ends; c. Outer surface of calyculus and margin connecting with the peridial net; d. Collapsed spore with warts as spore surface ornamentation. Scale bars: a = 100 µm; b = 5 µm; c = 50 µm; d = 1 µm.

the mutually exclusive conditions of that character." Four components for character statements were recognized: locators, variable, variable qualifiers, and characters states. All of this information can be expressed in symbolic form (for more details see Sereno 2007: Table 4).

The rationale for defining character and character states (character conceptualization), therefore, is a crucial step in evolutionary morphology. In order to render the information obtained with the use of SEM for the genus *Cribraria*, we have applied the scheme proposed by Sereno (2007) for morphological characters with a symbolic connotation. Here we summarize the data obtained for the specimens analyzed whose phylogenetic information could be tested through a cladistics framework. These results were interpreted as suggesting that a character has at least two different character states (variables feature), and considering all the range variation observed for the species included in the present study (see Table 1, p. 416).

Table 1. Symbolic notation for characters and character states in sporotheca and spores of the *Cribraria* species.

Character (locator)	Character states (variables)	Symbolic notation*
	Sporotheca	
Peridial net	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Peridial ribs	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Calyculus	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Nodes differentiated	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Thickness of nodes	(0) Pulvinate; (1) flattened	$L_1: V_0 ; V_1$
Type of nodes in peridium	(0) Enlarged; (1) isodiametric; (2) irregular	$L_1, L_2: V_0; V_1; V_2$
Calyculus upper margin	(0) Entire; (1) irregular; (2) ribbed; (3) Toothed	$L_1, L_2: V_0; V_1; V_2; V_3$
Calyculus surface	(0) Radial folds; (1) concentric folds	$L_1, L_2: V_0; V_1$
	Spore surface ornamentation	
Warts	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Crests	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Reticulum	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Bands	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Spiny-subreticulate	(0) Absent; (1) present	$L_1: V_0 ; V_1$
Grooved-reticulate	(0) Absent; (1) present	$L_1: V_0 ; V_1$

*L_1 = primary locator, L_2 = secondary locator; V_n = character state

Conclusions

In this paper, we have described spore ornamentation variables (character states) as revealed by SEM never before noted for species such as *C. ferruginea* and *C. laxa*. Studies of micro-morphological characters in *Cribraria* with SEM, such as spore ornamentation and the shape of nodes, demonstrate that interpretations of these character states based on LM alone may be incorrect or completely lacking. This is because LM does not make certain features prominent or even noticeable in the same way as SEM. *Cribraria*, as observed by Ing (1999) and Adamonyte & Eliasson (2010), is a challenging genus due to overlapping morphological variation among the various species, making it problematical to circumscribe particular features with accuracy. Therefore, SEM is a fundamental tool for taxonomic studies in this genus as well as for other myxomycete taxa for which similar empirical difficulties exist. Further SEM research in *Cribraria*, either of same species or those not included in this work, will certainly confirm or amplify the morphological variation noted here.

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Glossary

Basal disc: A small portion of the calyculus remaining at the base of the sporotheca.

Calyculus: The lower portion of the sporotheca remaining as a persistent structure like a cup (Keller & Braun 1999)

Nodes: "The thickened area at the point of juncture where several threads meet to form the peridial network in the upper part of the sporotheca" (Keller & Braun 1999).

Peridial net: A persistent peridium that remains as a reticulate structure in the sporotheca (from Martin & Alexopoulos 1969).

Peridial ribs: Subparallel elements of the peridium, interconnected by short transverse threads, which may arise from the base of the sporotheca or extend from the calyculus margin.

Plasmodial granules: Microscopic, usually dark-colored structures found in the sporotheca of *Cribraria*, on either the peridial nodes or calyculus. These have been called "dictydine granules" (Martin & Alexopoulos 1969; Keller & Braun 1999) or "lime globules" (Nannenga-Bremekamp 1991). We prefer to use the term "plasmodial granules" based on their plasmodial origin.

Spore ornamentation: The surface of the spore, as seen under light microscope, may be smooth or have variously formed projections. Ornamentation features include:

- **Bands:** Flat (generally wide) regions that typically cross to form a reticulate pattern.
- **Crests:** projections distributed in waves.
- **Grooved-reticulate:** a net-like pattern (reticulum) formed by deep grooves or furrows.
- **Reticulum:** the pattern produced by warts distributed in lines that cross in a net-wise fashion.
- **Warts:** short obtuse projections distributed over the surface.